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A Non-invasive Isotopic Approach to Estimate the Bone Lead Contribution to Blood in Children: Implications for Assessing the Efficacy of Lead Abatement

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Abbreviations:

Pb_{blood}	Concentration of lead in blood, in units of μg Pb/dL blood
Pb_{feces}	Lead excretion in feces, in units of μg Pb/day
Pb_{in}	Amount of lead in blood from external intake, in units of μg Pb/dL blood
Pb_{bone}	Amount of lead in blood from bone, in units of μg Pb/dL blood
$(^{207}Pb/^{206}Pb)_{blood}$	$^{207}Pb/^{206}Pb$ isotopic ratio measured in blood
$(^{207}Pb/^{206}Pb)_{feces}$	$^{207}Pb/^{206}Pb$ isotopic ratio measured in feces
$(^{207}Pb/^{206}Pb)_{in}$	$^{207}Pb/^{206}Pb$ isotopic ratio of lead in blood from external intake
$(^{207}Pb/^{206}Pb)_{bone}$	$^{207}Pb/^{206}Pb$ isotopic ratio of lead in blood from bone
t_1, t_2, t_3	Time of the first, second, and third sample collection round, respectively
SRM	Standard Reference Material
RSD	Relative Standard Deviation

Outline

ABSTRACT

Lead hazard control measures to reduce children's exposure to household lead sources often result in only limited reductions in blood lead levels. This may be due to incomplete remediation of lead sources, and/or to the remobilization of lead stores from bone, which may act as an endogenous lead source that buffers reductions in blood lead levels. Here, we present a non-invasive isotopic approach to estimate the magnitude of the bone lead contribution to blood in children following household lead remediation. In this approach, lead isotopic ratios of a child's blood and five-day fecal samples are determined before and after a household intervention aimed at reducing the child's lead intake. The bone lead contribution to blood is estimated from a system of mass balance equations of lead concentrations and isotopic compositions in blood at the different times of sample collection. The utility of this method is illustrated with three cases of children with blood lead levels in the range 18 to 29 μg/dL. In all three cases, the release of lead from bone supported a substantial fraction of the measured blood lead level post-intervention, up to 96% in one case. In general, the lead isotopic compositions of feces matched or were within the range of the lead isotopic compositions of the household dusts with lead loadings exceeding EPA action levels. This isotopic agreement underscores the utility of lead isotopic measurements of feces to identify household sources of lead exposure. Results from this limited number of cases support the hypothesis that the release of bone lead into blood may substantially buffer the decrease in blood lead levels expected from the reduction in lead intake.

Introduction

The majority (>70%) of the body lead burden in children is contained within the skeleton (Barry 1981). Since lead is qualitatively a biologic analog to calcium, its uptake and release from the skeleton are partly controlled by processes affecting bone growth and turnover (Hu et al. 1998; O'Flaherty 1998). In adults, skeletal lead is contained within long-lived compartments of cortical (elimination $t_{1/2}$ > 5 - 10 years) and trabecular (elimination $t_{1/2}$ > 1 year) bone, with comparatively small amounts of lead in tissue compartments that rapidly exchange with extracellular fluid and plasma (Hu et al. 1998; Leggett 1993; O'Flaherty 1998; Rabinowitz et al. 1976). In children, however, the turnover rates of skeletal reservoirs of lead and the impact of bone lead releases on blood lead content, are not well understood. In children exposed to lead hazards, the accumulation of lead in bone and other tissues is of serious concern because these body lead stores are believed to serve as internal sources of lead to blood during bone remodeling (Gulson et al. 1996; Gulson et al. 1997b; Leggett 1993; Nordberg et al. 1991; O'Flaherty 1994). Moreover, mobilization of accumulated skeletal lead stores back into blood is suspected to be responsible for the apparent limited success of various lead hazard control measures to decrease blood lead levels in exposed children (Burgoon et al. 1995; Rust et al. 1999). These abatement efforts typically result in reductions of blood lead levels in exposed children of no more than 30% when evaluated within several months after intervention (US EPA 1995).

The importance of bone lead storage and mobilization in controlling blood lead levels has been documented in adults (Rabinowitz et al. 1976; Smith et al. 1996). Increased bone resorption in winter months (Oliveira et al. 2002), during pregnancy

and lactation (Gulson et al. 1997a; Lagerkvist et al. 1996; Manton et al. 2003; Rothenberg et al. 2001; Silbergeld 1991; Tellez-Rojo et al. 2002), under hyperthyroidism conditions (Goldman et al. 1994), or due to skeletal disease (Berlin et al. 1995) has been associated with elevated blood lead levels. In addition, experimental lead isotope studies in non-human primates have demonstrated lead releases from bone to blood (Inskip et al. 1996). The existence of a relationship between bone remodeling and blood lead content has been also hypothesized for children (Angle et al. 1995; Gulson et al. 1996; Gwiazda and Smith 2000; Hu et al. 1998; Manton et al. 2000; O'Flaherty 1994; Rust et al. 1999). Yet such a link has been difficult to document partly because of the challenges of studying pediatric populations, including the use of non-invasive K-x-ray fluorescence techniques to assess bone lead burdens in children (Hu et al. 1998).

Measurements of bone lead content in children could be used to establish empirical relationships between bone and blood lead levels in pediatric populations. However, this relationship would be affected by other factors such as current lead intake, age, and history of exposure that are thought to affect the nature of the bone lead – blood lead relationship. Nevertheless, because of the importance of bone lead in human lead toxicokinetics, the potential effect of bone turnover on blood lead content has been included in the structure of pharmacokinetic models of childhood lead poisoning. These include the Integrated Exposure and Uptake Biokinetic model, IEUBK (White et al. 1998), the O'Flaherty physiologically based pharmacokinetic model (O'Flaherty 1998), and Leggett's biokinetic lead model (Leggett 1993).

Validation of these models for bone lead release has been limited, however, because of the scarcity of suitable pediatric data for accurate ground-truthing and calibration.

Lead isotopic methods provide an alternative approach to estimate the impact of endogenous sources of lead on blood lead content (Gulson et al. 1997b; Smith et al. 1996). In its simplest form, this approach apportions the blood lead isotopic composition as a mixture of two end members, the lead isotopic composition of intake and the lead isotopic composition of the endogenous source(s). The critical challenge in the application of this approach is the characterization of the isotopic composition of the end members contributing lead to blood (e.g., external sources and the skeleton). To this end, a variety of experimental designs has been utilized in adults. In these designs, the isotopic composition of the lead intake was either estimated from analysis of environmental samples (Gulson et al. 1995; Gulson et al. 1996; Manton 1985; Smith et al. 1996), and from duplicate diets (Gulson et al. 1997a; Manton 1985; Rabinowitz et al. 1976), or was purposefully changed (Rabinowitz et al. 1973; Rabinowitz et al. 1976; Rabinowitz et al. 1977; Facchetti 1989). The lead isotopic composition of the endogenous skeletal source was estimated on the basis of the assumed historical exposure (Gulson et al. 1995; Gulson et al. 1997b) or measured directly on bone samples (Manton 1985; Smith et al. 1996). In some cases the fraction of lead derived from the skeleton was calculated from simple proportionality (Gulson et al. 1995; Smith et al. 1996) while in others its computation required the utilization of mathematical models (Colombo and Fantechi 1983; Rabinowitz et al. 1973; Rabinowitz et al. 1976; Rabinowitz et al. 1977).

Studies by Gulson and colleagues (Gulson et al. 1996; Gulson et al. 1997b), who utilized the lead isotopic approach to estimate the skeletal lead contribution to blood in children, took advantage of the fact that the studied children had lived at a younger age in locations with a presumably well characterized environmental lead isotopic composition, which was different from that of their current exposure. It was then assumed that the lead in their skeleton carried a homogeneous isotopic signature from the earlier exposure. While their work provides supporting evidence for the contribution of bone lead to blood lead in children, their methodological approach is limited to the special circumstances of a child moving to a very different location. In most cases, however, the lead isotopic composition of children's skeletal tissue cannot be empirically ascertained, and therefore the apportionment of the blood lead isotopic composition between the skeletal and the exogenous end-members is not possible. Isotopic measurements of shed deciduous lead in teeth could serve as proxy of the lead isotopic composition of bone, but this opportunistic sampling would only be useful for children aged 5 and older. Similarly, it is often difficult to obtain a weighted average lead isotopic composition of lead intake, given the various possible sources and pathways of exposure (dust, soil, food, air) from which children absorb lead. Isotopic characterization of lead intake has been done from duplicate diets. However, this sampling method does not include all sources of lead exposure to the child, especially for younger children who may ingest high amounts of lead from environmental sources due to increased hand to mouth activity.

Here, we present a non-invasive isotopic approach to estimate the magnitude of the bone lead contribution to blood in children following household lead remediation. .

This approach does not require lead isotopic measurements of bone nor does it assume the lead isotopic ratio of bone on the basis of the child's lead exposure history. Instead, blood and feces are sampled for lead concentration and isotopic analyses before and after the implementation of environmental lead hazard control measures to reduce the child's lead exposure(s). Estimation of the bone lead contribution to blood using this method is illustrated with three cases of childhood lead poisoning. In addition, the sources of lead exposure to the child are identified from a comparison of the lead isotopic compositions of household sources and feces, using the latter as a surrogate measure of the magnitude and isotopic composition of lead intake.

Materials and Methods

Experimental Approach

The isotopic composition of blood is a function of the isotopic compositions and relative lead contributions of exogenous intake and endogenous sources. Here, it is assumed that the isotopic composition of feces reflects the isotopic composition of lead intake. However, the isotopic composition of bone is not known. In order to calculate this value and be able to solve the relative lead contributions from intake and from the skeleton to blood, we rely upon an induced change in the magnitude and isotopic composition of lead intake through the elimination of identified household sources of lead exposure (i.e., household lead abatement intervention). The assumption in this approach is that by reducing the magnitude of the child's lead intake, the relative contribution of lead to blood from the endogenous skeletal source increases and the lead isotopic composition of blood shifts towards the isotopic value of bone.

A system of linear equations was applied to calculate the endogenous lead contribution to blood, with blood and feces lead content and isotopic composition before and after intervention as independent variables. More generally, this system of equations can be applied between any two time points with different blood lead levels, regardless of the cause and direction of change in blood lead content (increase or decrease).

We used the following mass balance equations for lead content and for lead isotopes at two different time points, t_1 and t_2, to describe the mixing of lead in blood:

$$Pb_{blood}^{t_1} = Pb_{in}^{t_1} + Pb_{bone}^{t_1} \quad (1)$$

$$Pb_{blood}^{t_2} = Pb_{in}^{t_2} + Pb_{bone}^{t_2} \quad (2)$$

$$(^{207}Pb/^{206}Pb)_{blood}^{t_1} * Pb_{blood}^{t_1} = (^{207}Pb/^{206}Pb)_{in}^{t_1} * Pb_{in}^{t_1} + (^{207}Pb/^{206}Pb)_{bone}^{t_1} * Pb_{bone}^{t_1} \quad (3)$$

$$(^{207}Pb/^{206}Pb)_{blood}^{t_2} * Pb_{blood}^{t_2} = (^{207}Pb/^{206}Pb)_{in}^{t_2} * Pb_{in}^{t_2} + (^{207}Pb/^{206}Pb)_{bone}^{t_2} * Pb_{bone}^{t_2} \quad (4)$$

where Pb_{blood} is the concentration of lead in blood, in units of μg/dL. Pb_{in}, and Pb_{bone} are the amounts of lead in blood from external intake and bone, respectively, in units of μg/dL. The $(^{207}Pb/^{206}Pb)_{in}$ is the isotopic ratio of lead in blood derived from external intake, and it is assumed to be identical to the isotopic composition of lead measured in feces, as follows:

$$(^{207}Pb/^{206}Pb)_{in}^{t_1} = (^{207}Pb/^{206}Pb)_{feces}^{t_1}$$

$$(^{207}Pb/^{206}Pb)_{in}^{t_2} = (^{207}Pb/^{206}Pb)_{feces}^{t_2}$$

It is also assumed that the lead isotopic composition of bone did not change between the two time points considered, as follows:

$$(^{207}Pb/^{206}Pb)_{bone}{}^{t_1} = (^{207}Pb/^{206}Pb)_{bone}{}^{t_2} = (^{207}Pb/^{206}Pb)_{bone.}$$

It is assumed that the amount of lead in blood from the external intake, Pb_{in}, is proportional to the rate of lead intake. Here, the rate of lead excretion ($Pb_{feces,}$ in units of μg Pb/day) is used as a surrogate of the rate of lead intake, as follows:

$$Pb_{in}{}^{t_1} = K * Pb_{feces}{}^{t_1} \quad (5)$$

$$Pb_{in}{}^{t_2} = K * Pb_{feces}{}^{t_2} \quad (6)$$

where K is a biokinetic constant that relates the lead content of feces to the amount of lead in blood from external intake. This biokinetic constant K is different from the more familiar term biokinetic slope factor (BKSF) that refers to the increase in blood lead per unit of lead *absorbed* (instead of excreted) across the GI tract (Bowers et al. 1994; Bowers and Cohen 1998; US EPA 2003). This approach yields a solvable system of six equations with six unknowns, K, $Pb_{in}{}^{t_1}$, $Pb_{in}{}^{t_2}$, $Pb_{bone}{}^{t_1}$, $Pb_{bone}{}^{t_2}$, $(^{207}Pb/^{206}Pb)_{bone,}$ with no need for parameterization (e.g., use of independently obtained parameters that describe fractional lead absorption across the GI tract, rate of bone turnover, etc.).

Notably, this method can be applied to obtain the relative contributions of lead from the intake and the skeleton to blood only when the lead isotopic compositions of the

external (intake) and internal (skeleton) sources are different. In practical terms, since the isotopic composition of the skeletal lead source typically cannot be known, this approach can be applied only if the difference between the blood and intake lead isotopic compositions are greater than the isotopic measurement error.

Subjects

Children were recruited by the Children's Hospital of Philadelphia from referrals by the Philadelphia Childhood Lead Poisoning Prevention Program (CLPPP). Inclusion criteria were: the blood lead level was between 15 μg/dL and 35 μg/dL, the child was less than 6 years of age, the child spent most of his/her waking time within a single household environment, and the blood and fecal lead isotopic compositions were measurably different (to confirm the latter criteria, blood and fecal samples were analyzed within two weeks following recruitment). Four cases were recruited. Three boys, ages 14, 20, and 46 months, met the inclusion criteria and were retained in the study (Table 1). One case did not meet the latter criteria above and was excluded from follow-up. Informed written consent was obtained from all parents/guardians. All procedures used in the recruitment of subjects, including the administered questionnaire and the collection of biological and environmental samples received prior review and approval by the human subjects institutional review boards of the University of California at Santa Cruz and the Children's Hospital in Philadelphia.

Sample Collection

Blood, five-day complete fecal samples, and household environmental samples were collected at enrollment. Within two months, a state certified lead abatement

contractor conducted a thorough cleaning of the household environment, including HEPA vacuuming and wet washing of all horizontal surfaces with tri-sodium phosphate detergent. Blood and five-day fecal samples were collected a second time (t_2) at least one month after the household cleaning. Finally, a third round of blood and five-day fecal collections were performed at least three months after the second round of sample collection (Table 1).

Blood samples (3mL) were collected into low-lead heparinized vacutainer tubes (#367734, Becton-Dickinson, Franking Lakes, NJ) by the Children's Hospital of Philadelphia. Parents collected daily fecal samples in diapers provided by the study (LUVS Ultra Leak Guards #4) or in perforated urine collection "hats" (McKesson Medical Surgical, Richmond, VA) that had been pre-washed with distilled water and air-dried in a filtered-air environment. Household samples of all deteriorated paints, floor dusts and soils, if appropriate, were sampled by the Philadelphia Health Department following HUD guidelines (HUD 1995). All samples were shipped to University of California, Santa Cruz for lead concentration and isotopic composition analyses, as described below.

Analytical techniques

Processing of biological samples was conducted under trace-metal clean HEPA filtered air (Class-100) conditions using clean techniques (Smith et al. 1992). Acids used in sample processing and analyses were quartz double distilled, water was ultra-pure grade (18 MΩ cm^2), and all sample processing plastic-ware was acid-cleaned (Flegal and Smith 1992).

Blood samples were processed in triplicate as described in Gwiazda and Smith (2000). Ultra-pure water was added to fecal samples (at least 2 parts water: 1 part feces, w/w), and the mix homogenized with a stainless steel blender. Duplicate ~2.5 gram aliquots of the fecal homogenate were dried and digested overnight in 2-3 mL sub-boiling 16N HNO_3. After evaporation to dryness, samples were reconstituted in 1N HNO_3, and centrifuged at 15,000 x g. The supernatant was spiked with ^{209}Bi for analysis in an inductively coupled plasma mass spectrometer (ICP-MS), as described below.

Paint (0.1-0.2 g) and soil (~1 g) samples were homogenized with mortar and pestle, weighed, and digested in trace metal grade 16N HNO_3 for at least 12 hours. After evaporation to dryness, samples were reconstituted in 1N HNO_3, filtered (Whatman filter paper #4) and spiked with ^{209}Bi for analysis by ICP-MS. Dust wipes were digested in a similar fashion.

A double focusing magnetic sector ICP-MS (Element, Finnigan MAT) was used for lead isotopic and concentration measurements using the method of Gwiazda et al. (1998), but with shorter scan times of 10 ms for each mass. ^{204}Pb abundance was not measured. National Institute of Standards and Technology standard reference material 955b level 4 (lead in blood) was used to evaluate the precision of lead isotopic and accuracy of lead concentration measurements in blood. The measured lead concentration of the 955b blood SRM was 38.6±1.3 μg/dL (2 x standard error, SE, n=5), in good agreement with the certified value of 39.4 μg/dL. The precision of the blood $^{207}Pb/^{206}Pb$ ratio measurements over the course of the study was 0.2% (2 x relative standard deviations, RSD), based on the analyses of NIST 955b blood SRM

over five different days of analyses. The precision of blood $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratio measurements within an analytical run was 0.16% and 0.26% (2 x RSD) respectively, based on triplicate analyses of the children's blood samples at each single collection interval. The average difference in $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios between homogenized feces duplicates was 0.11% and 0.16%, respectively (n = 39 pairs). Precision and accuracy of lead isotopic ratios of environmental samples was estimated from repeated measurements of NIST981 (common lead isotopic standard reference material). The long-term precision of NIST981 $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios was 0.13% and 0.10% (2 x RSD, n = 5 different measurement days) and the accuracy was within 0.05% of the certified ratio values.

Diaper blank was estimated to be ~5.8ng lead per diaper, based on the analyses of ultra-pure water rinsed over the inner surface of new diapers (n=12). Fecal sample contamination associated with homogenization was <5 ng lead, based on total procedural homogenization blanks (n=6) processed with each batch of feces. These lead blank values are three orders of magnitude less than the typical amount of lead found in feces in a diaper, indicating that fecal lead contamination associated with collection and processing was negligible.

Results

Relationships between lead isotopic ratios of environmental, feces and blood samples

This study was able to distinguish analytically the various household sources of environmental lead, since the overall range of $^{207}Pb/^{206}Pb$ ratios of environmental

samples from all households (i.e., 4%) was about 20-times larger than the isotope ratio measurement error (<0.2%) (Figure 1). In general, the lead isotopic compositions of feces from the first round of sampling (i.e., prior to household intervention) match (cases 1 and 2, Figure 1A and 1B) or are bracketed (case 3, Figure 1C) by the lead isotopic compositions of the household dusts with lead loadings exceeding EPA action levels that were collected in the same visit.

The lead content of feces of case 3 in the first visit indicates very variable daily lead intake (Figure 1C). The highest daily fecal lead content, 240 μg Pb, is up to 30-times higher than the average daily fecal lead content of ~6 μg Pb/day of the children in the other two cases. A lead content-weighted grand average fecal isotopic composition for each sampling round was calculated based on the lead content and isotopic composition of the daily fecal samples (Figure 2). These calculations indicate that the isotopic compositions of blood and average feces of case 3 (Figure 2C) from the first sampling round (t_1) are in much closer proximity to each other than what is observed in the other two cases, consistent with a greater relative impact of recent lead exposures on blood lead levels.

In contrast to case 3, the lead intakes of cases 1 and 2 in the first round of sampling (t_1) are low, as reflected in the lead content of feces (~6.5 and 5 μg/day, respectively) (Figure 1A and 1B). However, the blood lead levels in these two cases (20.3 and 29.3 μg/dL, respectively) are comparable to or higher than in case 3 (18.3 μg/dL). In addition, in both cases 1 and 2, blood from all sampling rounds contained higher $^{207}Pb/^{206}Pb$ ratios than the average feces (Figure 2A and 2B).

Blood lead levels declined between the first (t_1) and second (t_2) visits in all three cases, even though in cases 1 and 2 there was no significant change in the fecal lead content (Figure 2A, 2B). In these two cases the blood isotopic composition at the second visit (t_2) moved towards the isotopic composition of feces (case 1) or remained unchanged (t_2, case 2).

In contrast to this is case 3, where the fecal lead content decreased post-intervention (t_2) and the blood $^{207}Pb/^{206}Pb$ ratios shifted away from the isotopic composition of feces (Figure 2C). When the lead content of feces again increased at the final round of sampling (case 3, t_3), the blood lead level also increased (from 12.9 to 16.6µg/dL at t_2 and t_3, respectively and its isotopic composition shifted back closer to the fecal lead $^{207}Pb/^{206}Pb$ ratio (Figure 2C). No significant change was observed in case 2 between t_2 and t_3.

Estimates of bone lead contribution to blood

Estimates of the amount of lead in blood from bone at each collection time point are obtained by applying equations (1) though (6) (see Methods) to blood and fecal lead concentrations and isotopic ratios of any two sampling rounds (i.e., t_1 and t_2, t_1 and t_3, t_2 and t_3). Thus, in cases 2 and 3, where three sampling rounds took place, it is possible to obtain two estimates of the bone lead contribution to blood for each collection time point. This is because each sampling round is used in two different pairs of time points in the calculations. For example, two estimates of bone lead contribution to blood were calculated for t_2, one estimate based on the t_1 and t_2 sample collection pair, and the other based on the t_2 and t_3 sample collection pair (Table 2).

The estimated bone lead contribution to blood in the oldest child (46 months age, case 2) is consistent throughout the three sampling rounds and amounts to more than 90% of blood lead. In other words, uptake of lead from external sources in that child supported less than 10% of the lead in blood throughout the 7.4 months encompassed by the sampling rounds. Since blood lead levels in that child ranged between ~25 – 29 μg/dL throughout the study, these results indicate that the chronically elevated blood lead levels may be attributed to the mobilization of substantial bone lead stores. In case 1, where only two sampling visits took place, the estimated bone lead contribution averaged ~65% but decreased slightly from the first visit (73%) to the second visit (58%), consistent with the reduction in blood lead levels (from 20.3 to 14.9μg/dL) and with the absence of a reduction in fecal lead elimination (i.e., lead intake) over the time interval (Table 1, 2, Figure 2A).

In case 3, the amount of lead in blood from bone is more variable. For this case, the estimates of the bone lead contribution to blood on the basis of the two different sampling pairs are 36% at t_1 (average of 19 and 53% calculated using the t_1-t_2 and t_1-t_3 collection times, respectively), 65% at t_2 (average of 59 and 70%, from pairs t_1-t_2 and t_2-t_3, respectively), and 40% at t_3 (average of 33 and 48% from pairs t_1-t_3 and t_2-t_3, respectively). This variability in the estimates of bone lead contribution calculated using two different sampling pairs is due to the relative size of the analytical measurement error compared to the isotopic differences between blood and feces from two collection time points. When isotopic differences between samples collected at different times are small, the measurement uncertainty in the isotopic values results in large

uncertainties in the estimates of all parameters calculated from equations (1) through (6).

The biokinetic factor K (equations 5 and 6) relates the amount of lead in blood from external intake with the lead content of feces, i.e., with the amount of lead ingested but not absorbed. If it is assumed that gastrointestinal (GI) absorption of lead in infants and small children is on the order of 50% (Alexander et al. 1974; Ziegler et al. 1978), the value of K should be numerically equivalent to the more commonly used biokinetic slope factor (BKSF) defined as the increase in blood lead per unit of lead absorbed across the GI tract (Bowers et al. 1994; Bowers and Cohen 1998; US EPA 2003). Data from infants fed formula mixed with leaded water (Sherlock and Quinn 1986) suggest a BKSF for small children of 0.21-0.11 in the blood range 13 μg/dL to 30 μg/dL, if GI lead absorption is assumed to be 0.5. Calculated biokinetic factors (K) (Table 2) range from 0.83 to 0.12, though they are generally much more consistent for a given child. Comparison of biokinetic factors (K) across children, and even across studies should be done with caution since this term is not normalized to body weight.

Discussion

The three case studies presented here serve to illustrate the application of this non-invasive isotopic approach to estimate the bone lead contribution to blood in lead poisoned children. These results substantiate that in lead exposed children reductions in blood lead levels post-intervention may be buffered by the release of significant amounts of lead from bone into blood, and thus may not adequately reflect reductions in lead exposure from environmental sources. The endogenous source of this lead

mobilized into blood is presumed to be the skeleton, since the skeleton contains the majority of the body lead burden. Thus, the ability of a household lead abatement intervention to produce considerable reductions in the blood lead level of a chronically lead exposed child may be substantially limited by the large contribution of bone lead to blood.

This is best demonstrated by case 2, the oldest child examined here (46 months age at enrollment), whose fraction of lead in blood from bone was calculated to be >90% throughout the study. Supporting this, the lead intake of this child was comparatively low (~6 μg/day fecal lead elimination) and constant throughout the three sampling visits, yet the blood lead level was very elevated and decreased only slightly over time (29.3 down to 25.2 μg/dL over 7 months). Thus, even if the lead intake had been completely eliminated by the household abatement intervention, the expected decrease in blood lead level would have been very small. This case, in particular, illustrates the limitations of assuming that blood lead levels are direct indicators of current environmental lead exposure, and that lead hazard control measures would necessarily be efficacious in significantly reducing blood lead levels.

In cases 1 and 3, the estimated bone lead contribution to blood was calculated to be smaller than in case 2 (i.e., ~40 – 65% Vs >90%) and, at least in case 3, more variable over time. The different estimated contributions of bone lead to blood lead over time in the three children studied here could be due to a number of factors, including differences in exposure history and levels of lead accumulated in bone. The child with the largest bone lead contribution to blood (case 2, >90%) was the oldest of the three children and had a very low lead intake, based on fecal lead elimination. In

this case, a larger store of bone lead accumulated over a prolonged period (i.e., years) of exposure to elevated environmental lead levels could have maintained elevated blood lead levels that only very slowly decreased over time once the exposures were controlled. Under this scenario, reduction of the elevated environmental exposures to the case 2 child may have occurred prior to the conduct of this study, consistent with his relatively low fecal lead content at enrollment. In the other two cases of younger children (~1.5 years old), the bone lead contribution to blood was smaller and more variable (at least in case 3), suggesting a smaller reservoir of lead in bone, possibly due to a shorter history of environmental exposure.

Historically, the efficacy of lead abatement practices for reducing childhood lead exposures has been evaluated based on reductions in blood lead levels as indicators of lead exposure/uptake (Aschengrau et al. 1994; Aschengrau et al. 1998; Charney et al. 1983; CDC, 1991; Farfel and Chisolm 1990; Hilts et al. 1995; Kimbrough et al. 1994). This approach does not sufficiently consider the very important contribution of accumulated bone lead stores in 'buffering' reductions in blood lead levels post-intervention. Though it may be more difficult to verify, a more accurate appraisal of the effectiveness of lead hazard control measures would be based on their success in reducing lead intake.

Fecal lead content measured over several days is one possible approach to estimating the overall magnitude of childhood lead intake. Fecal lead content should give an integrated measure of lead exposure/intake from all sources, dietary and environmental, inside and outside the home. In contrast, other approaches such as duplicate diet sampling may not sufficiently reflect total lead exposure/intake because

duplicate diets do not reflect potentially important environmental sources of lead to children living in older housing or in the proximity of soils with high lead content. Similarly, hand-wipes may provide an estimate of non-dietary environmental lead exposure in some cases (Duggan et al., 1985), though they do not reliably reflect ingestion of environmental lead.

There are, however, limitations with the use of fecal lead content as a measure of lead intake. First, collection of complete fecal samples over multiple days may not be feasible in some cases. Second, variability among children in GI lead absorption should ideally be taken into consideration if fecal lead content were to be used as a direct surrogate of lead uptake and intake. Third, because excreted fecal lead reflects unabsorbed ingested lead in addition to lead eliminated via endogenous fecal (e.g., biliary) routes, variation in these physiologic processes from child to child may introduce variation not attributable to environmental lead exposure. Nonetheless, fecal lead content still may be the among the most accurate indicators of the amount and isotopic composition of lead the child is ingesting and as a result, it may serve as a useful quantitative index of the extent of oral lead exposure from all sources (diet and environment).

Assumptions and Limitations of this Isotopic Approach

The mathematical approach utilized here to calculate the bone lead contribution to blood relies on a number of assumptions, including: 1) The lead isotopic composition of feces reflects the lead isotopic composition of ingested lead that is incorporated into the circulation, and; 2) The isotopic composition of the skeleton remains constant throughout the 3 – 6 month interval between consecutive sampling

visits. Although prior studies have not systematically validated the first assumption, it is supported by a number of published observations. Studies where lead intake and excretion were measured in animals and humans showed that an increase in lead intake is quickly followed by an increase in fecal lead excretion (Barltrop and Killala 1967; Kehoe 1987; Ziegler et al. 1978). In addition, fecal lead excretions by children suffering elevated lead exposures have been shown to correlate with the degree of lead paint hazards in their household environment (Hammond et al. 1980). Perhaps most relevant to the present study, Rabinowitz (1987) showed that the lead isotopic compositions of leaded paints in the household environments of lead poisoned children matched the isotopic compositions of the children's bloods and their excreted feces. These latter observations are replicated in the three cases reported here where the lead isotopic compositions of feces matched or were within the range of the lead isotopic compositions of the household dusts with the highest lead loadings.

An additional factor related to the first assumption that is not included in the model, but that may effect the utility of fecal lead content as a surrogate of lead intake, is the elimination of lead into feces via endogenous (e.g., biliary) pathways. Biliary lead excretion has been shown to range between 40 to 85 % of total body lead excretion in non-human primates (O'Flaherty et al. 1996; Cremin et al. 2001), less than 46% in adult humans (Rabinowitz et al. 1976), and it is estimated to be at least 50% in infants (Ziegler et al. 1978). Assuming that the lead isotopic composition of bile matches that of blood, the biliary elimination of endogenous lead into feces would shift the lead isotopic value of feces towards the isotopic value of blood, though the extent of this shift would depend on the amount (e.g., µg) of lead eliminated from this biliary route

compared to the amount of lead in the feces (i.e., unabsorbed lead from oral intake). Consequently, the measured difference in lead isotopic composition between feces and blood would be smaller than the true isotopic difference between blood and the oral intake. Here, we conservatively chose to not include in the model a term for endogenous biliary lead excretion into feces, since endogenous fecal lead excretion is unknowable for a particular child, and including this term in the model would introduce a large uncertainty in the calculation of the bone lead contribution to blood. Functionally, the impact of not including this variable in the model produces calculated bone lead contributions to blood that, if anything, are *minimum* values that could be larger for a particular child.

The second assumption of this model is that the isotopic composition of the skeleton remains constant throughout the sampling visits (i.e., the model assumes $(^{207}Pb/^{206}Pb)_{bone}^{t_1} = (^{207}Pb/^{206}Pb)_{bone}^{t_2}$). It is possible though that changes in the blood isotopic composition because of reductions in lead intake following intervention for example, could produce small changes in the isotopic composition of metabolically active regions of bone that exchange lead with blood. Accordingly, the bone lead isotopic composition could change slightly towards that of blood, rather than remain constant throughout the study. If allowance is made in the model for a change in bone isotopic composition towards that of blood, as the magnitude of external sources of exposure change, the estimated contribution of lead from bone to blood actually *increases*. Thus, the approach utilized here, which assumes that the bone lead isotopic composition does not change with time, yields a *minimum* calculated value for the contribution of bone lead to blood.

Conclusions

A non-invasive isotopic approach is presented to estimate the bone lead contribution to blood in children following interventions to reduce environmental lead exposures. Illustration of this method using three cases of lead poisoned children provides evidence that mobilized skeletal lead stores may contribute a significant fraction of lead to blood, up to 90% or more in one case presented here, which may substantially 'buffer' reductions in blood lead levels following environmental lead remediation. Since the accumulated skeletal lead burden likely varies from child to child, depending in part upon the child's age and lead exposure history, it should be expected that blood lead levels would decrease at different rates post-intervention, depending on the contributions of bone lead to blood in different children. This suggests that the efficacy of lead hazard remediation efforts should be evaluated over prolonged periods (i.e., >6 – 12 months or more) to allow adequate time for depletion of accumulated skeletal lead stores and a reduction in their absolute contribution to blood lead levels. Observations from this study also support the use of fecal lead content and isotopic composition as a proxy for the identification of sources of lead exposure.

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Table 1: Age in months and blood lead level (BPb) of children at enrollment, household lead abatement, and subsequent sample collection visits.

	Enrollment (t_1)		Abatement	2^{nd} collection (t_2)		3^{rd} collection (t_3)	
	Age	BPb	Age	Age	BPb	Age	BPb
Case 1	14 mo,	20.3 µg/dL	15.2 mo.	16.1 mo,	4.9 µg/dL	Withdrew[a]	
Case 2	46 mo,	29.3 µg/dL	47 mo.	49 mo,	25.4 µg/dL	53.4 mo,	25.2µg/dL
Case 3	20 mo,	18.3 µg/dL	22.1 mo.	27 mo,	12.9 µg/dL	36 mo.,	16.6 µg/dL

[a]Child withdrew from the study prior to the 3^{rd} visit.

Table 2: Calculated fraction of lead in blood from bone, $^{207}Pb/^{206}Pb$ of bone, and biokinetic factor, from equations (1) through (6)

		Sampling round		
Case		**t_1**	**t_2**	**t_3**
1	Fraction of lead in blood from bone (%)	73%	58%	NA
	Bone lead isotopic composition	0.8610	0.8610	
	Biokinetic factor K, (μg /dL)/(μg /day), eq. (5)	0.83	0.83	
2	Fraction of lead in blood from bone[a] (%)	91-92%	91-96%	92-96%
	Bone lead isotopic composition[a]	0.8737 - 0.8750	0.8742 - 0.8750	0.8737 - 0.8742
	Biokinetic factor K[a], (μg /dL)/(μg /day), eq. (5)	0.48 - 0.14	0.21 - 0.48	0.14 - 0.21
3	Fraction of lead in blood from bone[a] (%)	19 - 53%	59 - 70%	33 - 48%
	Bone lead isotopic composition[a]	0.8474 - 0.8517	0.8505 - 0.8517	0.8474 - 0.8505
	Biokinetic factor K[a], (μg /dL)/(μg /day), eq. (5)	0.12 - 0.21	0.15 - 0.21	0.12 - 0.15

[a]In cases 2 and 3, two values can be calculated for each time point by pairing data from each sample collection time with data from either one of the other two collection times (see Methods). For example, for t_1 equations 1 through 6 can be applied in combination with t_2 or in combination with t_3.

Figure Captions:

Figure 1: Lead isotopic ratios of blood, feces, and environmental samples, and household dust lead loadings from the first visit (t_1). The vertical bar below each dust isotopic composition symbol (blue circle) is the lead loading of that particular dust sample according to the right ordinate scale. Numbers adjacent to the feces symbols (brown squares) indicate the day of the 4 - 5 day sequential feces sample collection. Panel A: Case 1, fecal lead content (μg): day 1 = 7.0, day 2 = 6.6, day 3 = 2.9, and day 4 = 9.0. Panel B: Case 2, fecal lead content (μg): day 1 = 5.1, day 2 = 6.0, day 3 = 4.7, day 4 = 1.9, and day 5 = 7.0. Panel C: Case 3, fecal lead content (μg): day 1= 4.7, day 2 = 41, day 3 = 240, and day 4 = 7.3.

Figure 2: Lead content and isotopic composition of blood (circles) and feces (squares) from Case 1, 2, and 3 (Panel A, B, and C, respectively). The visit number (1^{st}, 2^{nd}, or 3^{rd}) is included inside the symbols. The fecal lead isotopic composition shown is the lead content-weighted average of the lead isotopic compositions of the daily feces collected in the visit. The black rectangle along the X-axis is the range in calculated isotopic composition of lead in the skeleton.

Figure 1

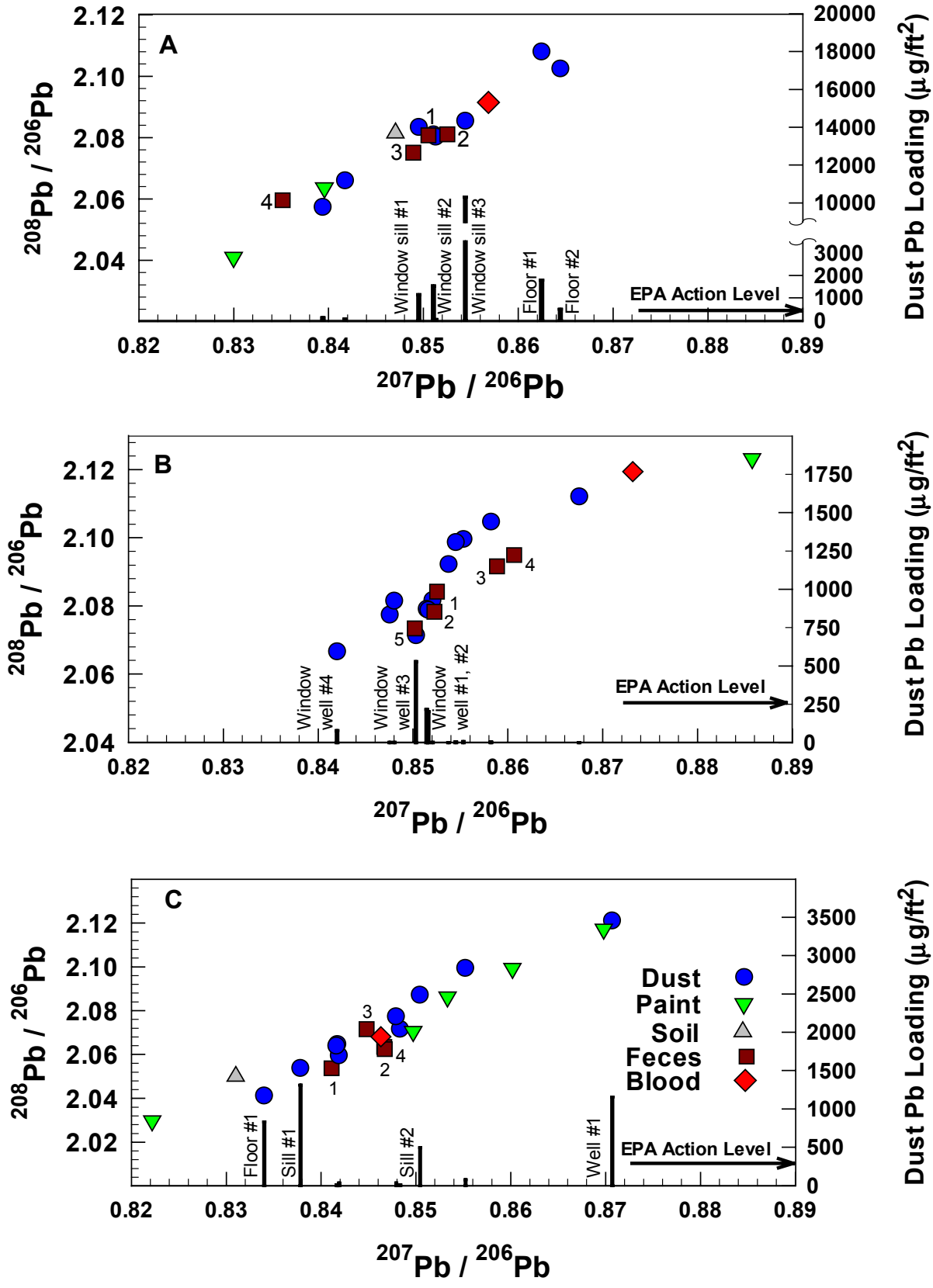
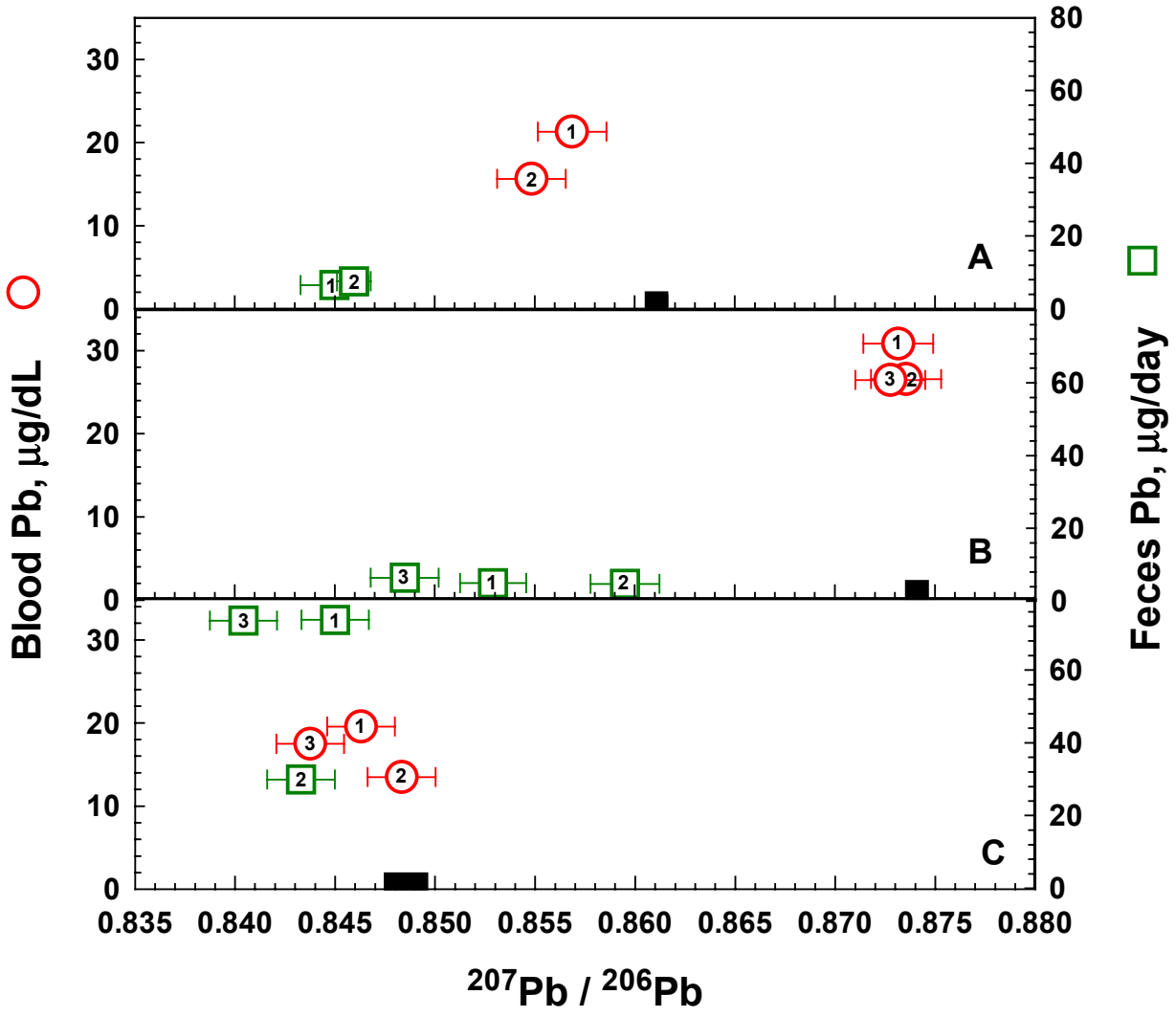

A
B
C
^{208}Pb / ^{206}Pb
^{207}Pb / ^{206}Pb
Dust Pb Loading (µg/ft^2)
EPA Action Level
Window sill #1
Window sill #2
Window sill #3
Floor #1
Floor #2
Window well #4
Window well #3
Window well #1, #2
Sill #1
Sill #2
Well #1
Dust
Paint
Soil
Feces
Blood

Figure 2


Blood Pb, µg/dL
Feces Pb, µg/day
A
B
C
0
10
20
30
40
60
80
0.835
0.840
0.845
0.850
0.855
0.860
0.865
0.870
0.875
0.880
207Pb / 206Pb